Submission form for phytosanitary treatments

(Reviewed by TPPT March 2016)

Czech Republic, Central Institute for Supervising and Testing in Agriculture (UKZUZ)

Click here to find the IPPC Procedure Manual for Standard Setting on the IPP (www.ippc.int), where you can download this form.

Submission number (Secretariat Use Only):

Complete the following form, preferably in electronic format, and submit by e-mail to the IPPC Secretariat (ippc@fao.org). The call will remain open, but if you wish your submission to be considered by the TPPT in their next meeting, please send it before the 5 June 2017.

Please use one form per phytosanitary treatment. An electronic version of this form is available on the International Phytosanitary Portal (IPP) at https://www.ippc.int/en/publications/1089/. Incomplete submissions will be returned. Please save the completed submission form with the following file name: COUNTRY or RPPO NAME –Title of treatment.doc, prior to submitting to the IPPC Secretariat via e-mail. The words "Call for Phytosanitary Treatments" should be placed in the subject line of the email message.

(Text in brackets given for explanatory purposes)

Name of treatment	*Hydrogen cyanide fumigation treatment for Ditylenchus dipsaci in seed bulbs of garlic*

Submitted by: Central Institute for Supervising and Testing in Agriculture (UKZUZ), Czech Republic

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Treatment description

Active ingredient	Hydrogen cyanide (HCN)
Treatment type	Chemical - fumigation
Target pest	*Ditylenchus dipsaci*
Target regulated articles	Seed bulbs of garlic
Treatment schedule	Active substance- hydrogen cyanide (HCN) in preparation "Uragan D2" (new trade name BLUEFUME, hereinafter BLUEFUME).

	BLUEFUME is a mixture (stabilized liquid hydrogen cyanide) of approx. 98 % of hydrogen cyanide with stabilizing additives. Fumigation is performed by using of pressure cylinders. Composite pressure cylinders are consisted of stainless steel 316L liner and fiberglass overwrap. Cylinders are equipped with stainless steel 316L dual port valve with dip tube for liquid HCN outlet and gas port for nitrogen pressuring. Elastomeric sealing is from polychlorotrifluoroethene (PCTFE). The cylinders contain up to 27.5 kg of HCN. BLUEFUME is produced and filled into a cylinder as liquid. Due to fumigation conditions (application by spraying nozzles, temperature, pressure), the liquid evaporates (and does not condense back) and so the active substance is gas. Use in fumigation chamber Application rate: 20 g/m^3 Fumigation temperature and time: 24 °C; 14 hrs Ventilation time: min 24 hrs Timing the treatment during the 4–6 week period after harvest Efficacy: 99% Ventilation through alkali cleaning The fumigation may be performed only by specifically trained professional personnel.
Other relevant information	In the previous EPPO protocols (e.g. EPPO 1974), hydrogen cyanide (HCN) was available for fumigation treatment of bulbs, rhizomes and tubers against *D. dipsaci*. That EPPO protocol was withdrawn in 1985, and was substituted with the protocol nr. PM 3/2(2), which is the fumigation of seed material infested with *D. dipsaci* by methyl bromide (EPPO 1998). Registration according EU 528/2012 as a biocidal preparation "BLUEFUME" for usage: PT08, PT14 and PT18.
References	05_HCN_ZOUHAR_garlic_2016 Safety data sheets for BLUEFUME – cylinders Validation report confirms the suitability of GC method in laboratory conditions.

The following form must be completed in accordance with ISPM 28 *Phytosanitary treatments for regulated pests*, the IPPC Strategic Framework and the *Procedure and criteria for identifying topics for inclusion in the IPPC standard setting work programme*.

Copies of all relevant supporting information and publications should be supplied with the treatment submission, preferably in PDF format, for ease of subsequent distribution.

The following form refers to the relevant sections of ISPM 28 and are numbered accordingly.

3.2 Efficacy data in support of the submission of a phytosanitary treatment

Efficacy data are based on laboratory study which was published in form of article "Using of hydrogen cyanide against *Ditylenchus dipsaci*".

Overview of all support submitted documents is attached to this form.

3.2.1 Efficacy data under laboratory/controlled conditions (Treatments may be considered without efficacy data under laboratory/controlled conditions if sufficient efficacy data is available from the operational application of the treatment (section 3.2.2) and if no data under laboratory/controlled conditions exists this section may be left blank.)
Pest information
Identity of the pest to the appropriate level, life stage, and if a laboratory or field strain was used
Ditylenchus dipsaci
Conditions under which the pests are cultured, reared or grown
Naturally (*D. dipsaci* were obtained from a farm in Central Bohemia)
Biological traits of the pest relevant to the treatment
X
Method of natural or artificial infestation
natural infestation

Determination of most resistant species/life stage (in the regulated article where appropriate)
X
Regulated article information
Type of regulated article and intended use
Garlic seedlings
Botanical name for plant or plant product (where applicable)
Allium sativum- cultivars *Vekan*, *Bjetin*, *Blanin*, *Benátčan*, *Stanik*, *Slavin*, Jovan*, Japo II* and *Matin*
Conditions of the plant or plant product
X
Experimental parameters
Level of confidence of laboratory tests provided by the method of statistical analysis and the data supporting that calculation
Obtained data of the study underwent statistical analysis (two way and one-way ANOVA, regression analysis; Statistica 12, StatSoft, Inc., Tulsa, USA, 2013). The results of the biological tests were evaluated in cooperation with: Czech University of Life Sciences Prague Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources Kamýcká 957 Prague - Suchdol Czech Republic - 165 00 and Crop Research Institute (CRI) Drnovská 507 Prague 6 Czech Republic - 161 06
Experimental facilities and equipment
All experiments were performed in a fumigation chamber localised within the Draslovka Kolín a.s. Unique hermetical fumigation chamber (size 120x90x60cm, made of stainless steel sheets, (material 17240) with a thickness of 2 mm); gas chromatograph (Shimadzu GC-17A, RT-QPLOT, 30 m, ID 0.53 mm,GC Software Clarity DataApex, Kyoto, Japan); statistical analysis (two way and one-way ANOVA, regression analysis; Statistica 12, StatSoft, Inc., Tulsa, USA, 2013); common laboratory equipment.
Experimental design
HCN fumigation of garlic clove naturally infested by *D. dipsaci*. The study was designed to determine: 1) Penetration of HCN into garlic tissue The study was conducted in a hermetic fumigation chamber. Garlic for testing was prepared by hollowing the single cloves of garlic and attaching a rubber septum onto the hollow area. Treatments were conducted in a stainless hermetic steel fumigation chamber equipped with an air lock, forced ventilation and rubber glove manipulators. The injected dosage of HCN in the head space of the fumigation chamber was equivalent to 20 g/m^3. HCN samples were withdrawn from the garlic cloves via the septum using a glass syringe and the HCN concentrations were determined using a gas chromatograph after 6, 12, 18, 24 and 30 h of exposure; the concentration of HCN inside chamber was also measured. Result: The HCN concentration in the core of the garlic cloves was approximately 30% of the initial concentration inside the chamber headspace after 30 h of treatment. Strong and simple relationship of exposure time and concentration of HCN inside garlic tissue (correlation coefficient 0.98) as well as in fumigation chamber (correlation coefficient 0.90) enabled estimation of equalizing of concentrations in both environments at 50 h of hypothetical treatment. 2) HCN phytotoxicity A second experiment targeted the evaluation of the phytotoxic effect of HCN on seed garlic cloves. The design of this experiment was similar to the previous one. Nine cultivars of garlic were obtained and treated with HCN (concentration 20 g/m^3) for 10 exposure periods. Seven cloves were treated in each cultivar. After treatment, garlic cloves were planted and numbers of emerging plants were scored 30 days after planting. Untreated control variants of each cultivar were included in the experiment.

Result: Our tests on phytotoxicity further showed that HCN did not affect the viability of garlic in the short treatments (up to 14 h); however, there was a decrease in germination after longer HCN exposures.

3) Biological efficacy on D. dipsaci

Garlic cloves infested with *D. dipsaci* were obtained from a farm in Central Bohemia. The presence and quantification of the species was confirmed using a Baermann funnel extraction technique. To determine the location of the nematodes within the cloves, 56 cloves with peels removed were examined and the removed peels were examined separately. On average, one clove contained 7422 *D. dipsaci* specimens- 7.406 in peels and 16 in the bare clove. Ten infested cloves were inserted into fabric sacks (mesh size 45 µm) for fumigation. Five replicates were treated for each cultivar. The injected dosage of HCN in the head space of the fumigation chamber was equivalent to 20 g/m^3. The temperature inside the chamber was maintained at 24°C during the trials. The three exposure times tested were 12, 18 and 24 h, and HCN samples were also withdrawn from the chamber at those intervals. After 24 h, the HCN was ventilated from the samples, and surviving nematodes were extracted from treated cloves using the Baermann funnel technique and counted under a stereomicroscope. An untreated control was placed beside the fumigation chamber during the trials and was evaluated in the same manner. Obtained data underwent statistical analysis (two way and one-way ANOVA, regression analysis; Statistica 12, StatSoft, Inc., Tulsa, USA, 2013).

Result: Overall, there was good efficacy of the HCN treatment on *D. dipsaci* mortality (i.e. 99%). Even the shortest treatment period significantly decreased the number of nematodes in the garlic tissue. No significant differences were observed among the three exposure times. After all three tested exposure times, some living nematodes were extracted; however, the number of survivors was only approximately 1% of the numbers recorded in the untreated control.

Experimental conditions
Dosage: 20 g/m^3 of HCN; temperature: 24°C; fumigation time: depends on experiment; sampling: depends on experiment (see above)
Monitoring of critical parameters
Concentration HCN in fumigation chamber was measured by gas chromatograph (Shimadzu GC-17A, RT-QPLOT, 30 m, ID 0.53 mm,GC Software Clarity DataApex, Kyoto, Japan)
Methodology to measure the effectiveness of the treatment
Surviving nematodes were extracted from treated cloves using the Baermann funnel technique and counted under a stereomicroscope. Obtained data underwent statistical analysis (two way and one-way ANOVA, regression analysis; Statistica 12, StatSoft, Inc., Tulsa, USA, 2013).
Determination of efficacy over a range of critical parameters, where appropriate
All critical parameters were followed.
Methodology to measure phytotoxicity, when appropriate
Nine cultivars of garlic were obtained and treated with HCN (concentration 20 g/m^3) for 10 exposure periods. Seven cloves were treated in each cultivar. After treatment, garlic cloves were planted and numbers of emerging plants were scored 30 days after planting. Untreated control variants of each cultivar were included in the experiment.
Dosimetry system, calibration and accuracy of measurements,
Accuracy of measurement of above-mentioned gas chromatograph is ± 0,05 vol. %
3.2.2 Efficacy data using operational conditions (historical data, may in some cases substitute for the requested information below)
Pest information
Identity of the pest to the appropriate level, life stage, and if a laboratory or field strain was used
X
Conditions under which the pests are cultured, reared or grown
X
Biological traits of the pest relevant to the treatment
X
Method of natural or artificial infestation
X
Determination of most resistant species/life stage (in the regulated article where appropriate)
X
Regulated article information
Type of regulated article and intended use
X
Botanical name for plant or plant product (where applicable)
X
Conditions of the plant or plant product
X
Experimental parameters

Level of confidence of laboratory tests provided by the method of statistical analysis and the data supporting that calculation
X
Experimental facilities and equipment
X
Experimental design
X
Experimental conditions
X
Monitoring of critical parameters
X
Methodology to measure the effectiveness of the treatment
X
Determination of efficacy over a range of critical parameters, where appropriate
X
Methodology to measure phytotoxicity, when appropriate
X
Dosimetry system, calibration and accuracy of measurements
X
Factors that affect the efficacy of the treatment
X
Special procedures that affect the success of the treatment, if applicable
X
3.3 Feasibility and applicability (Information should be provided where appropriate on the following items)
Procedure for carrying out the phytosanitary treatment
Fumigation is provided by a professional pest control company licensed for professional fumigation. A pesticidal registration has not been completed yet but results of this study show the potential of possible exceeding intended uses of hydrogen cyanide. Approved by the National Authority for Biocide Registration as a biocidal preparation of the type: PT08,14 and 18
Cost of typical treatment facility and operational running costs if appropriate
The price is not available, however, it may be provided later following an agreement with a professional company carrying out fumigation.
Commercial relevance, including affordability
Costs of using hydrogen cyanide are lower than of using methyl bromide.
Extent to which other NPPOs have approved the treatment as a phytosanitary measure
X
Availability of expertise needed to apply the phytosanitary treatment
x
Versatility of the phytosanitary treatment
The preparation BLUEFUME (active substance hydrogen cyanide) is registered as a biocide in 13 states of EU, but is fully adaptable to the usage for phytopathological treatment. In terms of its originality – efficiency, capacity and rate of the application process, BLUEFUME with hydrogen cyanide as the active substance has no comparable and acceptable alternative regarding the health protection (substance without CMR effects on humans), environmental protection and social-economical aspects. BLUEFUME is in selected areas of usage a fully effective substitute for methyl bromide. HCN is not classified as substance hazardous to the ozone layer, and is a very important strategic material for the Czech Republic. BLUEFUME is the only fully effective substitute for methyl bromide in the above mentioned usages.
The degree to which the phytosanitary treatment complements other phytosanitary measures
Due to simplicity of use and high efficacy to many species and developmental stages of various pests is usage seeming like a full-fledge substitute of methyl bromide. Moreover due to smaller structure of molecule HCN offers more possible field of application.
Summary of available information of potential undesirable side-effects
Human exposure may occur by inhalation of hydrogen cyanide vapours or absorption through the skin upon contamination of work clothing by vapours or liquid hydrogen cyanide. The risk of exposure applies to workers in the production when exposure limits are exceeded, or in case of accident.

Workers carrying out fumigation shall use protective equipment; exposure should not occur if operating procedure is observed.
Bystanders or other persons may not be exposed to hydrogen cyanide, since it is intended only for professional use in empty secured spaces.
Due to the form in which hydrogen cyanide is supplied – pressure cylinder – accidental exposure of employees or other persons is beyond consideration.

Professional exposure
Since hydrogen cyanide is applied as gas, its main route of entry is inhalation and dermal exposure.
HCN is intended to be used for fumigation – to control insect pests and smaller rodents in closed structures and insect pests damaging various natural materials (in gas-tight application chambers).

If used professionally as intended, no adverse impacts to humans or animals, except target organisms, are expected.

Risks for employees in production, fumigation workers and bystanders
The risk of inhalation of hydrogen cyanide during production and processing is minimised by automatic monitoring of the workplace atmosphere and signalisation of danger if permissible exposure limits are exceeded. Employees shall use specified personal protective equipment.

Workers carrying out fumigation shall use protective equipment and personal detectors and are bound to strictly observe specified operating procedures.

Hydrogen cyanide is intended for authorised professional use only. It may be neither sold nor provided in any other way to unauthorised persons, including unauthorised persons from among professional users. The initial exclusion zone is set to protect bystanders and other once-in-a-lifetime exposed persons (limit 3 mg/m^3). If specified organisational measures, ensuring that non-professional users will not come into contact with HCN as the result of uncontrolled entry into fumigated area, or ingestion of food or drinks treated with HCN, are observed, exposure of non-professional users does not occur. In consequence of absent possibilities of exposure, hydrogen cyanide as fumigant does not present any real risk to the health of non-professional users.

Applicability of treatment with respect to specific regulated article/pest combinations

Almost 100% effective against all species of seedling (garlic, onion,..) insect pests as well as other pests occurring individually or in various combinations. Almost 100% effective was observed against *Caenorhabditis elegans* and *Bursaphelenchus xylophilus*.

Technical viability

BLUEFUME shall be stored in dry, cool, ventilated, separate storeroom. Due to the danger or accidental release of HCN, only personnel authorised to handle HCN may enter the storeroom, and only with a gasmask with suitable filter and a measuring device.
Keep container tightly closed.
Use explosion-proof electrical/ventilating/light/equipment. Take precautionary measures against static discharge.
Shelf life
At site practice storage stable. No decomposition. The shelf-life of BLUEFUME is set to 12 months.

Phytotoxicity and other effects on the quality of regulated articles, when appropriate

The exposures up to 14 h with a HCN concentration of 20 g/m^3 seem to be relatively safe in terms of phytotoxicity.

Consideration of the risk of the target organism having or developing resistance to the treatment

Due to toxicologist properties of HCN, by this time, no case of resistance has been proven.

Send submissions to:
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(preferred)

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